UBE1 [6His-tagged]

E1 - Ubiquitin Activating Enzyme

Alternate Names: A1S9, A1S9T, BN75 temperature sensitivity complementing, A1ST, CTD-2522E6.1, GXP1, MGC4781, tsA1S9, UBE1X, Ubiquitin-activating enzyme E1

Cat. No.	**61-0001-050**	Quantity:	50 µg
Lot. No.	**30010**	Storage:	-70˚C

FOR RESEARCH USE ONLY

NOT FOR USE IN HUMANS

Background

The enzymes of the ubiquitylation pathway play a pivotal role in a number of cellular processes including regulated and targeted proteasomal degradation of substrate proteins. Three classes of enzymes are involved in the process of ubiquitylation; activating enzymes (E1s), conjugating enzymes (E2s) and protein ligases (E3s). UBE1 is a member of the E1 activating enzyme family and cloning of the human gene was first described by Handley *et al*. (1991). The UBE1 gene has been mapped to Xp11.3-p11.23 by high-resolution fluorescence *in situ* hybridization (Takahashi *et al*., 1992). UBE1 'activates' ubiquitin through catalysing a C-terminal ATP dependent adenylation of the protein which results in it forming a high-energy thioester bond with the sulfhydryl group of UBE1. UBE1 is monomeric and there are two active sites within the UBE1 protein allowing it to bind two ubiquitin moieties at a time, with a new ubiquitin forming an adenylate intermediate as the previous one is transferred to the thiol site (Jin *et al*., 2007; Zheng *et al*., 2009). Defects in UBE1 are known to cause spinal muscular atrophy X-linked type 2 (SMAX2) also known as X-linked lethal infantile spinal muscular atrophy, distal X-linked arthrogryposis multiplex congenita or X-linked arthrogryposis type 1 (AMCX1). Spinal muscular atrophy refers to a group of neuromuscular disorders charac-

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Physical Characteristics

Species: human

Source: Sf21 insect cell-baculovirus expression

Quantity: 50 µg

Concentration: 0.5 mg/ml

Formulation: 50 mM HEPES pH 7.5, 150 mM sodium chloride, 2 mM dithiothreitol, 10% glycerol

Molecular Weight: ~121 kDa

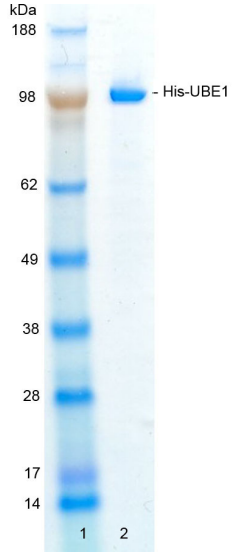
Purity: >98% by InstantBlue™ SDS-PAGE

Stability/Storage: 12 months at -70˚C; aliquot as required

Protein Sequence: Please see page 2

Quality Assurance

Purity:
4-12% gradient SDS-PAGE
InstantBlue™ staining
Lane 1: MW markers
Lane 2: 1 µg His-UBE1



Protein Identification:
Confirmed by mass spectrometry.

E1-Ubiquitin Thioester Loading Assay:
The activity of His-UBE1 was validated by loading ubiquitin onto the active cysteine of His-UBE1. Incubation of the His-UBE1 enzyme in the presence of ubiquitin and ATP at 30°C was compared at two time points, T_0 and T_{10} minutes. Sensitivity of the ubiquitin/His-UBE1 thioester bond to the reducing agent DTT was confirmed.

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Lot-specific COA version tracker: v1.0.0

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Background

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terized by degeneration of the anterior horn cells of the spinal cord, leading to symmetrical muscle weakness and atrophy. SMAX2 is a lethal infantile form presenting with hypotonia, areflexia, and multiple congenital contractures (Ramser *et al*., 2008).

References:

Handley PM, Mueckler M, Siegel NR, Ciechanover A, Schwartz AL (1991) Molecular cloning, sequence, and tissue distribution of the human ubiquitin-activating enzyme E1. *Proc Natl Acad Sci USA* **88**, 258-62.

Jin J, Li X, Gygi SP, Harper JW (2007) Dual E1 activation systems for ubiquitin differentially regulate E2 enzyme charging. *Nature* **447**, 1135-8.

Ramser J, Ahearn ME, Lenski C, Yariz KO, Hellebrand H, von Rhein M, Clark RD, Schmutzler RK, Lichtner P, Hoffman EP, Meindl A, Baumbach-Reardon L. (2008) Rare missense and synonymous variants in UBE1 are associated with X-linked infantile spinal muscular atrophy. *Am J Hum Genet* **82**, 188-93.

Takahashi E, Ayusawa D, Kaneda S, Itoh Y, Seno T, Hori T (1992) The human ubiquitin-activating enzyme E1 gene (UBE1) mapped to band Xp11.3----p11.23 by fluorescence in situ hybridization. *Cytogenet Cell Genet* **59**, 268-9.

Zheng M, Liu J, Yang Z, Gu X, Li F, Lou T, Ji C, Mao Y (2009) Expression, purification and characterization of human ubiquitin-activating enzyme, UBE1. *Mol Biol Rep* **37**, 1413-9.

Physical Characteristics

Continued from page 1

Protein Sequence:

M S Y Y H H H H H H D Y D I P T T E N L Y
F Q G A M G S ***S*** S S P L S K K R R V S G P D P K P G S N C
S P A Q S V L S E V P S V P T N G M A K N G S E A D I D E
G L Y S R Q L Y V L G H E A M K R L Q T S S V L V S
G L R G L G V E I A K N I I L G G V K A V T L H D Q G
T A Q W A D L S S Q F Y L R E E D I G K N R A E V S Q P R
L A E L N S Y V P V T A Y T G P L V E D F L S G F Q V
V V L T N T P L E D Q L R V G E F C H N R G I K L V
V A D T R G L F G Q L F C D F G E E M I L T D S N G E Q
P L S A M V S M V T K D N P G V V T C L D E A R H G F E S
G D F V S F S E V Q G M V E L N G N Q P M E I K V L G
P Y T F S I C D T S N F S D Y I R G G I V S Q V K V P K K
I S F K S L V A S L A E P D F V V T D F A K F S R P A Q L
H I G F Q A L H Q F C A Q H G R P P R P R N E E D A A E L
V A L A Q A V N A R A L P A V Q Q N N L D E D
L I R K L A Y V A A G D L A P I N A F I G G L A A Q E V M
K A C S G K F M P I M Q W L Y F D A L E C L P E D K E V
L T E D K C L Q R Q N R Y D G Q V A V F G S D L Q E K L
G K Q K Y F L V G A G A I G C E L L K N F A M I G L
G C G E G G E I I V T D M D T I E K S N L N R Q F L
F R P W D V T K L K S D T A A A A V R Q M N P H I R V T
S H Q N R V G P D T E R I Y D D D F F Q N L D G
V A N A L D N V D A R M Y M D R R C V Y Y R K P L L E S
G T L G T K G N V Q V V I P F L T E S Y S S S Q D P
P E K S I P I C T L K N F P N A I E H T L Q W A R D E
F E G L F K Q P A E N V N Q Y L T D P K F V E R T L R
L A G T Q P L E V L E A V Q R S L V L Q R P Q T W A D
C V T W A C H H W H T Q Y S N N I R Q L L H N F P P
D Q L T S S G A P F W S G P K R C P H P L T F D V N N P L
H L D Y V M A A A N L F A Q T Y G L T G S Q D R A A V A T
F L Q S V Q V P E F T P K S G V K I H V S D Q E L Q S A N
A S V D D S R L E E L K A T L P S P D K L P G F K M Y P
I D F E K D D D S N F H M D F I V A A S N L R A E N Y
D I P S A D R H K S K L I A G K I I P A I A T T T A A V
V G L V C L E L Y K V V Q G H R Q L D S Y K N G F L N
L A L P F F G F S E P L A A P R H Q Y Y N Q E W T L W
D R F E V Q G L Q P N G E E M T L K Q F L D Y F K T E
H K L E I T M L S Q G V S M L Y S F F M P A A K L K
E R L D Q P M T E I V S R V S K R K L G R H V R A L V
L E L C C N D E S G E D V E V P Y V R Y T I R

Tag (**bold text**): N-terminal His
Protease cleavage site: TEV (ENLYFQ▼G)
UBE1 (regular text): Start *bold italics* (amino acid residues 2-1058)
Accession number: NP_003325

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Lot-specific COA version tracker: v1.0.0